

Listing of Claims

The following listing of claims will replace all prior versions, and listings, of claims in the subject application:

1. (currently amended) A method for reproducibly generating dendritic cells, comprising the steps of:

(a) obtaining blood mononuclear cells through apheresis from G-CSF donors, with monocytes and monocyte precursors being separated substantially from red blood cells, platelets and lymphocytes, and loading the blood mononuclear cells into a cell culture container containing microcarrier beads and media for culturing dendritic cells therein, said media including rh-GM-CSF and at least one of rh-IL-4 or rh-IL-7 as ~~a~~-reagents;

(b) incubating the contents of the cell culture container, including the media and the blood mononuclear cells loaded in the container in step (a), in order to grow dendritic cell culture;

(c) removing nonadherent cells which do not adhere to the beads after the incubation in step (b), from the cell culture container, by resuspending the contents of the cell culture container, allowing the microcarrier beads in the container to settle, and expressing off the supernatant out of the container; and

(d) ~~further processing the contents of the cell culture container which remain in the container after step (c)~~ introducing additional media for culturing dendritic cells into the cell culture container after the nonadherent cells are removed from the cell culture container in step (c), said media including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-7;

(e) incubating the contents of the cell culture container after the additional media are introduced into the cell culture container in step (d); and

(f) agitating the contents of the container after the incubation in step (e); and

(g) harvesting dendritic cells from the contents after the contents are agitated in step (f).

2. (currently amended) A method for reproducibly generating dendritic cells, comprising the steps of:

(a) loading microcarrier beads and media for culturing dendritic cells into a cell culture container, said media including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-7 as reagents;

(b) obtaining blood mononuclear cells through apheresis from G-CSF donors, with monocytes and monocyte precursors being separated substantially from red blood cells, platelets and lymphocytes, and loading the blood mononuclear cells into the container;

(c) incubating the contents of the cell culture container, including the media and the mononuclear cells loaded in the container in step (b), in order to grow dendritic cell culture;

(d) removing from the cell culture container nonadherent cells which do not adhere to the beads after the incubation in step (b), by resuspending the contents of the cell culture container, allowing the microcarrier beads in the container to settle, and expressing off the supernatant out of the container; and

~~(e) further processing the contents of the cell culture container which remain in the container after step (d)~~
introducing additional media for culturing dendritic cells into the cell culture container after the nonadherent cells are removed from the cell culture container in step (d), said media including rh-GM-CSF and at least one of rh-IL-4 and rh-IL-7;

(f) incubating the contents of the cell culture container

after the additional media are introduced into the cell culture container in step (e);

(g) agitating the contents of the container after the incubation in step (f); and

(h) harvesting dendritic cells from the contents after the contents are agitated in step (g).

3. (original) The method of claim 1, wherein the container comprises a gas permeable cell culture bag.

4. (original) The method of claim 1, wherein the container is a closed vessel.

5. (previously presented) The method of claim 1, wherein step (c) further includes washing the blood mononuclear cells incubated in step (b) to remove nonadherent cells.

Claims 6-8 (canceled).

9. (original) The method of claim 1, wherein after step (c) samples are removed from the container for quality control.

10. (original) The method of claim 9, wherein the quality control includes at least one of viability staining, microbial analysis, cell enumeration, microscopic examination of dendritic cell morphology, and immunophenotyping to determine a purity of the dendritic cell preparation.

11. (original) The method of claim 1, wherein the blood mononuclear cells are obtained by apheresis.

Claims 12-13 (canceled).